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(54) Title: METHODS FOR TREATING INFLAMMATORY DISEASES

(57) Abstract: The present invention describes methods for the use of PPAR ligands in the treatment inflammatory endocrine, dermatological, cardiovascular immunological, neurological, ophthalmic, neoplastic, pulmonary diseases, and age-related dysregulations. In addition, methods are provided for treating said conditions and diseases comprising the step of administering to a human or an animal in need thereof a therapeutic amount of pharmacological compositions comprising a pharmaceutically acceptable carrier, and a PPARγ agonist which cross-activates PPARα or PPARδ or both, or a PPARγ partial agonist, or a PPARγ/RXR agonist, effective to reverse, slow, stop, or prevent the pathological inflammatory or degenerative process.

METHODS FOR TREATING INFLAMMATORY DISEASES

CROSS-REFERENCES TO RELATED APPLICATIONS

This application claims priority to US Provisional Patent Application No. 60/225,907 filed August 17, 2000 and US Provisional Patent Application No. 60/230,509 filed September 6, 2000.

FIELD OF THE INVENTION

This invention relates to use of PPAR (peroxisome proliferator-activated receptor)-γ ligands belonging to the group consisting of: 1) a pure PPARγ agonist, 2) a PPARγ agonist that also activates PPARα (PPARγ/PPARα dual agonist), 3) a PPARγ agonist that also activates PPARδ (PPARγ/PPARδ dual agonist), 4) a PPARγ agonist that also activates both PPARα and PPARδ (PPAR "pan-agonist"), 5) a PPARγ partial agonist, 6) a PPARγ/RXR agonist, leading to the transcription of its target genes controlled by these nuclear transcription factors. This invention relates to the uses of said ligands to treat diseases of multiple organ systems, including those contained in the cardiovascular system, integumentary system, skeletal system (cartilage and bone), bone marrow (myeloid and erythroid progenitor cells and cell lines), the immune system (including spleen, thymus, lymph nodes, Peyer's patch), central and peripheral nervous system, endocrine glands, exocrine glands, urogenital system, gastrointestinal system and pulmonary system.

Diseases and conditions that lead to impaired health derive from hereditary (genetic) or environmental insults (e.g. imposition of oxidative stress, exposure to toxins, carcinogens, mutagens, viral infective agent) in many instances, are a consequence of inappropriate or pathological function of PPARs. Evidence supporting a role of pathological function of PPARs have been demonstrated in the pathogenesis of chronic multigenic diseases involving pathological processes as they relate to: 1) derangements in intermediary metabolism, 2) inflammation with inappropriate induction of inflammatory genes, 3) inappropriate proliferation and neovascularization, 4) inappropriate tissue modeling, 5) abnormal phenotypic transition usually involving dedifferentiation, and 6) inappropriate apoptosis (programmed cell death). Pathological function of PPARs have also been shown to increased the risk for or susceptibility to chronic diseases and acceleration of the aging process.

Because PPAR isoforms are selectively (differentially) expressed in different tissues,

to different degrees (amounts expressed) and operationally intact (physiologically functional), the treatment of multigenic diseases require that the targeting ligand also be functionally pleiotropic in order to address the heterogenous components of these diseases. Hypertension, diabetes, congestive heart failure, psoriasis, atherosclerosis, dementia, demyelinating diseases, atopy, and the aging process are examples of such diseases and conditions. Similarly, this principle may be applied to isoform subtypes as in the case of the PPARγ subtypes, PPARγ1, PPARγ2 and PPARγ3.

The present invention relates to the development of compounds for the treatment (i.e. prevention, amelioration, attenuation, arrest or reversal) of a PPAR-dependent disease or condition. The disease process or condition may be associated with inappropriate expression of PPARs or dysfunctional PPARs. Or the pathology may be related to disruption(s) in signaling pathways of hormones, neurotransmitters or other informational molecules, wherein the PPAR target(s) is (are) intact. Consequently, ligands may be designed for repairing pathological lesions proximal to the PPARs or at the level of PPARs and their interaction with receptor co-activators and/or co-repressors.

In another aspect, application of this invention relates to the treatment (prevention, amelioration, attenuation, arrest or reversal) of "non-pathological" (non-life-threatening) conditions such as natural (normal) aging of organs and tissues conditions. Examples of methods relating to this aspect of the invention include preventing inappropriate tissue modeling through inappropriate expression of matrix metalloproteinases (MMPs) on the one hand, and promoting appropriate expression of matrix proteins such as collagen, laminin, elastin, proteoglycans, and tissue inhibitors of MMPs (TIMPs) on the other. The objective of these intervention would contribute to improving skin structure and function by increasing epidermal hydration, resilience, elasticity and functionality of skin.

Another aspect of the present invention relates to methods of screening libraries of compounds to determine which are the best candidates among the subject compounds for use in the practice of this invention.

BACKGROUND OF THE INVENTION

The PPARs consist of three closely related nuclear receptors, PPARα, PPARγ, PPARδ, encoded by three separate genes, and comprise a subfamily within the broader nuclear receptor superfamily (Mangelsdorf DJ, Evans RM. Cell 1995; 83:841–50). Nuclear receptors like PPAR possess DNA binding domains (DBDs) that recognize specific DNA sequences (called response elements) located in the regulatory regions of their target genes (see, Mangelsdorf, *et al.* Cell **83**:835-839 (1995)); Perlmann, *et al. Cell* **90**:391-397 (1997)). Activation of PPARs modulates the expression of genes containing the appropriate respective perixosome proliferator response elements (PPRE) in its promoter region. The three PPARs share about 80% and about 70% amino acid identity in their DBDs and ligand binding domains (LBDs), respectively. PPARα, the first PPAR to be cloned, is most highly expressed in the liver, kidney, heart, and muscle, and is activated by peroxisome proliferators, a structurally diverse group of compounds which includes fibrates, herbicides, and phthalate ester plasticizers (Reddy JK, Lalwai ND. Crit. Rev. Toxicol. 1983; 12:1–58) that cause marked proliferation in the size and number of peroxisomes in the livers of rodents, and is accompanied by hepatomegaly and risk for hepatocellular carcinoma. PPARα is the target for the fibrate class of hypolipidemic drugs, such as clofibrate, fenofibrate, and bezafibrate, which are used to lower triglycerides and raise HDL-cholesterol in dyslipidemic patients. In contrast relatively little is known about the biology of PPARδ, which is expressed at comparable levels in virtually all tissues. Interestingly, the PPAR/RXR heterodimers are permissive in that they can be activated by ligands for either PPAR or RXR. Although the DNA binding site for each of the PPAR/RXR heterodimers is a direct repeat of the consensus sequence, AGGTCA, separated by a single nucleotide spacer, a so-called DR-1 motif. However, there is evidence that the optimal response element differs slightly for each of the PPARs. These subtle differences in binding specificity together with the differences in tissue expression patterns undoubtedly contribute to the distinct physiological roles of the three PPAR subtypes. In contrast to PPARα, relatively little is known about the biology of PPARδ, which is expressed at comparable levels in virtually all tissues. All three PPAR subtypes bind to DNA as obligate heterodimers with RXRα, RXRβ, or RXRγ, the nuclear receptors for 9-cis-retinoic acid. In this respect, this invention specifically relates to the ligands of the PPARγ/RXR heterodimer.

To date, of the three isoforms, PPARγ is unique in that three different PPARγ transcripts, termed PPARγ1, PPARγ2 and PPARγ3, have been described, and are derived from the PPARγ gene by differential promoter usage. PPARγ1 is broadly expressed at moderate or low levels in almost all tissues, while PPARγ2, is restricted to adipose (white fat and brown fat) tissue and large gut, and PPARγ3 is confined to adipose tissue, macrophages and colonic epithelium in human tissues PPARγ1 is broadly expressed, albeit at low levels. Both PPARγ2 and PPARγ3 are highly expressed in adipose tissue, and PPARγ3 is also expressed in macrophages (Mangelsdorf DJ, Evans RM. Cell 1995;83:841-850; Spiegelman BM. Diabetes 1998;47:507-514; Willson TM, et al. J Med Chem 2000; 43:527-550). Throughout this writing PPARγ refers to PPARγ1, PPARγ2 or PPARγ3, or combination thereof.

PPARγ is functionally involved in intermediary metabolism of cells and tissues expressed therein. The PPARs are differentially expressed in different organs and tissues. PPARα and PPARδ tend to be ubiquitously expressed in most tissues. However, of the three, PPARδ is most highly expressed fetal tissue, placenta and in the brain and CNS, and may control differentiation in these tissues. Activation of PPARs modulate expression of genes involved in: 1) intermediary (e.g. glucose and lipid) metabolism, 2) regulation of mitosis and cell growth, 3) regulation of blood vessel growth (neovascularization), 4) regulation of cellular differentiation and phenotype transition, 5) immunoregulation (e.g. suppression of activated T lymphocytes) and the inflammatory response in cells of the immune system, and 6) regulation of apoptosis (programmed cell death). Inappropriate function of these processes lead to pathophysiolgical conditions involving metabolic (endocrine) dysfunction, proliferative diseases, inflammatory diseases, degenerative diseases, and carcinogenesis.

Unlike the classical steroid and retinoid receptors, the PPARs are remarkably promiscuous in their ligand binding properties. The molecular basis of this promiscuity is the presence of a large hydrophobic ligand binding site, more than 1000Å^3 in size. However, with significant differences in the shape of this cavity with respect to each PPAR isoform, a knowledge of their topographical features can be exploited in the design and development of isoform-selective PPAR ligands. Clustered at one corner of the ligand binding site are three tyrosine and histidine residues, which are involved in the mechanism of receptor activation through the formation of specific hydrogen bonds with the ligand that stabilize the AF2 helix. In the case of PPARγ, optimizing ligand interaction within the apoprotein by stabilizing the

AF-2 helix results in high affinity binding and receptor activation. PPARγ agonists are insulin-sensitizing agents which also promote adipocyte differentiation and adipogenesis with consequent weight gain. Stabilization of the AF-2 helix leads to activation of the genetic program for adipocyte differentiation and fat accumulation. However, it is possible to design a ligand capable of binding with high affinity to PPARγ without stabilizing the AF2 helix (Willson TM, et al. J Med Chem 2000; 43:527-550). An example of such a "partial agonist" is the compound, GW0072 inhibits adipocyte differentiation and adipogenesis but retains insulin-sensitizing, antidiabetic activity in animal models (Willson TM, et al. Annu Rev Biochem 2001; 70:341-367). These results suggest that the pathways of adipocyte differentiation and insulin sensitization can be pharmacologically dissociated. Recent evidence suggests that PPARδ is may be involved in lipid metabolism and myelination in the central nervous system. (Saluja I, et al. Glia 2001;33:191-204; Peters JM, et al. Mol Cell Biol 2000;20:5119-28; Oliver WR Jr, et al. Proc Natl Acad Sci USA 2001;98:5306-11).

PPAR agonists have been shown to inhibit the expression of inflammatory cytokines such as TNF-α, IL-1, IL-2, IL-6 in cells of the immune system including, T lymphocytes, B lymphocytes, monocytes, monocyte/macrophages, and splenocytes, and to suppress inflammation mediated by Th1 lymphocytes (Marx N, et al. J Immunol 2000;164:6503; Padilla J, et al. Ann N Y Acad Sci 2000;905:97; Clark RB, et al. J Immunol 2000;164:1364; Yang XY, et al. J Biol Chem 2000;275:4541). PPAR agonists has also been shown to inhibit proliferation and promote differentiation of a variety of normal and neoplastic cell types. Spiegelman et al., in Patent Cooperation Treaty application number PCT/US97/22879, published June 18, 1998, disclose methods for inhibiting proliferation of PPARγ-responsive hyperproliferative cells by using PPARγ agonists; numerous PPARγ agonists are disclosed by Spiegelman et al., as well as methods for diagnosing PPARγ-responsive cells.

A role for PPARs in the regulation of inflammation was suggested by studies showing that treatment of monocytes or macrophages with PPARγ agonists including 15d-PGJ2 and thiazolidinediones resulted in reduced expression of proinflammatory cytokines such as TNF-α and IL-6, and the inhibition of macrophage activation (for review, see: Willson TM, et al. Annu Rev Biochem 2001; 70:341-367). However, the concentrations of PPARγ agonists required to elicit these effects did not correlate well with those required to activate PPARγ in other cellular assays. Whereas several potent PPARγ agonists had no effect, 15d-PGJ2 was shown to be a potent inhibitor of cytokine production in monocytes or macrophages. These

data suggest that the anti-inflammatory effects of these potent non-prostaglandin PPARγ agonists might be mediated though a PPARγ-independent mechanism. Other studies support this hypothesis. For example, the potent PPARγ agonist, AD-5075 (5-[4-[2-(5-methyl-2-phenyl-4-oxazoly)-2-hydroxyethoxy]benzyl]-2,4-thiazolidinedione), and non-thiazolidinedione PPARγ/PPARα/PPARδ pan-agonist, L-796,449 (3-chloro-4-(3-(3-phenyl-7-propylbenzofuran-6-yloxy)propylthio)-phenylacetic acid), failed to suppress TNF-α and IL-6 cytokine production in lipopolysaccharide-treated mice (Thieringer R, et al. Immunol 2000; 164:1046-54). Moreover, several different PPARγ agonists, including 15d-PGJ2 and rosiglitazone, blocked cytokine production equally well in macrophages derived from either *PPARγ*+/+ or *PPARγ* -/- embryonic stem cells (Chawla A, et al. Nat Med 2001; 7:48-52). In direct contrast to these findings, activators of both PPARα and PPARγ agonists have been shown to inhibit or downregulate inflammatory molecules such as IL-2, inducible nitric oxide synthase (iNOS), and the proinflammatory nuclear transcription factors, NF-κB, AP-1, NFAT and STAT1. This controversial subject regarding the role of PPARs in suppression of inflammatory mechanisms has been reviewed by Delerive P, et al. J Endocrinol. 2001 Jun;169(3):453-9. These authors have suggested that an explanation of the conflicting findings may be explained by differential activation of co-activators and co-repressors that interact with individual PPAR isoforms and their interaction with pro-inflammatory transcription factors in different tissues. Moreover, the pro-inflammatory or anti-inflammatory effect, or lack of effect of a PPAR ligand (agonist) is likely to depend on the cellular phenotype expressed (phenotypic transition) in the respective pathological state or disease.

T lymphocyte activation is highlighted by the induction of IL-2 gene expression, which governs much of the early lymphocyte proliferation responses. The PPARγ ligands, troglitazone and 15d-PGJ2, but not PPARα agonist, Wy14643, inhibited mitogen-inducible IL-2 production proliferation in human peripheral blood T-cells. The effect was evident only in T lymphocyte cell lines expressing PPARγ and not the PPARγ-lacking cells. The activated PPARγ physically associates with transcriptional factor NFAT regulating the IL-2 promoter, blocking NFAT DNA binding and transcriptional activity. This interaction indicates an important immunomodulatory role for PPARγ ligands as immunotherapeutic drugs to treat T cell-mediated diseases by targeting IL-2 gene expression (Yang XY, et al. J Biol Chem 2000; 275:4541-4544).

SUMMARY OF THE INVENTION

This invention proposes that, by unknown and unpredictable mechanisms, ligand-activation of PPARγ, and PPARγ/RXR, through interactions with other nuclear factors, co-activators, co-repressors and components of immunomodulatory networking and signaling pathways, may be useful as therapeutic immunomodulating agents. The present invention generally relates to the treatment of T lymphocyte-related diseases that involve activation of NFAT, NF-κB or AP-1, inappropriate activation of nuclear transcription factors that regulate the transcription of genes encoding inflammatory cytokines, and inappropriate transcription of disease-dependent target genes. Examples of immunomodulatory cytokines relating to this invention include, interferon-γ(INFγ), tumor necrosis factor-α (TNF-α), IL-1β, IL-2, IL-6, IL-8, IL-10 (Chinetti G, et al. J Biol Chem 1998; 273:25573-25580; Escher P, Wahli W. Mutat Res 2000; 448:121-138; Ricote M, et al. J Leukoc Biol 1999; 66:733-739; Rocchi S, Auwerx J. Ann Med 1999; 31:342-351). This invention further relates to the prevention or treatment of disorders of inflammatory and immunomodulatory responses of the immune system involving: T helper (T_H), T suppressor (T_S), Th1 and Th2 lymphocytes, natural killer (NK) and other T cell subsets.

The methods described in this invention relates to an improvement in the prior art, related to superior efficacy of recruiting co-activators and/or co-repressors in order to optimize the activation or inactivation of the various PPAR isoforms in the treatment or prevention of a particular disease involving, for example: 1) promoting apoptosis in cells with the pathological phenotype, 2) blockade of inappropriate apoptosis induction in normal cells triggered by inflammatory molecules, in particular IL-2, and pro-inflammatory nuclear transcription factors, in particular NF-κB, as in neurodegenerative diseases (Alzheimer's disease) and autoimmune diseases (multiple sclerosis), 3) inhibiting systemic inflammation by suppressing Th1-mediated inflammatory cytokines and promoting Th1 to Th2 phenotypic transition, leading to treatment or prevention diseases such as atherosclerosis, syndrome X and congestive heart failure. An incomplete list of examples of diseases susceptible to the immunosuppressive effects of PPAR ligands, specifically a PPARγ agonist which cross-activates PPARα or PPARδ or both, or a partial PPARγ agonist, or a PPARγ/RXR ligand are: inflammatory skin diseases (e.g. psoriasis, atopic dermatitis, eczema, acne vulgaris, and other

dermatitides), neurodegenerative diseases (e.g. multiple sclerosis, Alzheimer's disease, Parkinson's disease), cardiovascular diseases (e.g. atherosclerosis, venous and arterial occlusive diseases, restenosis after invasive procedures, cardiomyopathy, myocardial fibrosis, congestive heart failure), angiogenesis and neovascularization in neoplastic and other diseases. The immune system includes T lymphocytes, B lymphocytes, monocytes, macrophages, monocyte/macrophages, macrophage-like cells (e.g. astrocytes in the brain, retinal pigmented epithelial cells in the retina), cells of myeloid origin in any tissue, in particular the bone marrow (stem cells, pre-promyelocytes, promyelocytes, myelocytes, granulocytes, plasma cells, mast cells, basophils, polymorphonuclear cells, eosinophils).

The activity of a particular PPAR isoform appears to depend on the degree to which the receptor protein is phosphorylated. It has been proposed that phosphorylation could alter interactions with important, as yet undefined, protein cofactors of PPARγ which act as corepressors or coactivators. In fact, it is now known that nuclear receptors function as "ligand-gated" platforms for the assembly of these cofactors into large protein complexes on specific DNA sequences (Spiegelman BM. Diabetes 1998; 47:507-514). Some of these coactivator proteins (CBP/p300, SRC1, pCAF) have histone acetyltransferase activity that functions to "open" the configuration of chromatin, allowing more efficient transcription. Others act as deacetylases which oppose the effects of acetyltransferases. Similar arguments apply to PPARα and PPARδ modulation of gene transcription. One theoretical problem is whether the nuclear receptor coactivators or corepressors identified to date are selective for particular PPAR receptors, and this remains unknown (Spiegelman BM. Diabetes 1998; 47:507-514). In fact, these coactivators or corepressors have multiple modes of action and hence, it is not clear which cofactors are more important for the function of any particular receptor (Puigserver P, et al. Science 1999; 286:1368-1371. It is also not obvious how the tremendous specificity of biological actions of the individual nuclear receptors are generated (Spiegelman BM. Diabetes 1998; 47:507-514). Consequently, the full spectrum of nuclear cofactors that regulate the transcriptional activity of the PPARs or PPAR/RXR heterodimers remain to be defined. Hence, it is impossible to predict with certainty the way in which a tissue expressing PPARγ, PPARα and/or PPARδ may respond to a particular ligand, or whether a particular pathological state will be attenuated, arrested, accentuated or worsened by said ligand. This is especially the case in which a single ligand activates more than one

PPAR isoforms Examples said compounds are described in this invention and in: Willson TM, et al. J Med Chem 2000; 43(4):527-50, see Table I), which is included in its entirety by reference, herein.

Thiazolidinediones are a class of oral insulin-sensitizing agents that improve glucose utilization without stimulating insulin release, are class of PPAR agonists. U.S. Patent No. 4,287,200, discloses certain thiazolidine derivatives having the ability to lower blood glucose levels. In addition, U.S. Patent No. 4,572,912, discloses thiazolindinedione derivatives having the ability to lower blood lipid and blood glucose levels. These compounds were shown to have the ability to decrease the levels of blood lipid peroxides, blood triglycerides and blood cholesterol. As described above, a PPARγ ligand that binds with high affinity but which inhibits adipocyte differentiation and adipogenisis while retaining its insulin-sensitizing activity is a PPARγ partial agonist (Oberfield, *et al.*, *Proc Natl Acad Sci USA* 96:6102-6 (1999)). Similarly, PPARα partial agonists may dissociate the antidylipidemic effects from the drug's propensity for peroxisome proliferation, thereby have the benefit of improving lipid metabolism with out the risk of hepatitis or myositis, established adverse effects of PPARα agonists such as the anti-dyslipidemic fibrates.

Recent discoveries suggest that the genes regulated by PPAR receptors also play a role in other processes. Binding of ligands to PPARs induce changes in the transcriptional activity of genes that modulate inflammatory processes, angiogenesis, cellular proliferation and differentiation, apoptosis, and the activities of iNOS, MMPases and TIMPs. These findings suggest that regulation of the action of PPAR may have a therapeutic role in treating diseases such as occlusive vascular diseases (e.g. atherosclerosis), hypertension, neovascular diseases (e.g. diabetic retinopathy), inflammatory diseases (e.g. inflammatory bowel disease and psoriasis), and neoplastic diseases (carcinogenesis).

The precise contribution of each particular PPAR subtype to transcriptional activation of particular genes is difficult to predict. DNA response elements for both PPARα and PPARγ have been found in the promoter regions of a variety of genes, including a number involved in lipid and fatty acid metabolism. For example, in fetal rat brown adipocytes, expression of the uncoupling proteins UCP-1, UCP-2 and UCP-3 is controlled via both PPARα and PPARγ activation. Activation of PPARγ elicited 5- and 3- fold increases in UCP-1 and UCP-3, respectively. In contrast, activation of PPARα increased UCP-1 ten-fold, but decreased UCP-1. Interestingly, when both PPAR and were activated, a synergistic

interaction occurred in regulation of UCP-3.

These differential and synergistic effects may be mediated by co-activator recruitment, suppression of co-repressor proteins, or direct interaction at the level of the PPRE (see, Teruel , *et al. Biochem Biophys Res Commun.* 273(2):560-4 (2000)). It is not known whether the nuclear receptor coactivators or corepressors identified to date are selective for particular PPAR receptors (see, Spiegelman, *et al.*, *Diabetes* 47:507-514 (1998)). Many coactivators or corepressors have multiple modes of action and hence it is not clear which cofactors are more important for the function of any particular receptor (see, Puigserver, *et al. Science* 286:1368-1371 (1999). Furthermore, the tremendous specificity of biological actions of the individual nuclear receptors (see, Spiegelman, *et al. Diabetes* 47:507-514 (1998)), strongly suggests that the full spectrum of nuclear cofactors that regulate the transcriptional activity of PPARγ and/or PPARα remains to be defined.

Due to this lack of understanding of PPARγ and PPARα-related activity and mechanisms, as well as the differential expression of PPARγ and PPARα in cells, it is difficult to ascertain the potential effects of concurrent activation of PPAR γ and α receptors on both cellular processes relevant to disease. For example, PPARα or PPARγ may either have similar or disparate effects. It is known that inflammatory activation of human aortic smooth-muscle cells is inhibited by PPARα, but not by PPARγ. Apoptosis in human monocyte-derived macrophages is induced by activation of either PPARα and PPARγ (see, Staels *et al. Nature* 393:790-3 (1998)); Chinetti, *et al. J Biol Chem.* 273:25573-80 (1998)). However, PPARγ activation by troglitazone or 15-deoxy-Δ-12-14-prostaglandin J2 protects cerebellar granule cells from cytokine-induced apoptotic cell death (see, Heneka, *et al. J Neuroimmunol* 100:156-68 (1999)).

To summarize, PPAR isoforms exhibit differential patterns of tissue expression, different actions on different response elements, differential effects on co-activators and co-repressors, and differential regulation of access to the core transcriptional machinery. This complexity of PPAR regulation makes it extremely difficult to predict precisely which genes will ultimately be activated (transcribed) or inactivated (suppressed) as a result of activation by a particular combination of an agonist or an antagonist of PPARγ or PPARα. As a consequence, it is impossible to predict with certainty the way in which a tissue expressing PPARγ and PPARα may respond to a particular ligand, or whether a particular pathological

state will be attenuated, arrested, accentuated or worsened by said ligand. This is especially the case in which a single ligand activates both PPARγ and PPARα to similar degrees.

In view of this complex interplay between PPARγ and PPARα, it is desirable to synthesize compounds which bind both receptors and can take advantage of potential synergistic effects. For example, PPARγ and PPARα activation has been shown to inhibit proliferation (see, Ellis, *et al. Arch Dermatol.* **136**:609-616 (2000)) and promote differentiation of epidermal keratinocytes, respectively (see, Komuves *et al. J Invest Dermatol.* **115**:353-360 (2000)). Furthermore, it is especially desirable to take advantage of potential of the potential for dissociating the beneficial and non-beneficial (pleiotropic) effects of a PPARγ agonist by utilizing the PPARγ partial agonist. An example of a genetic manifestation of this kind dissociation is evident in *PPARγ* +/− mice, which lack one copy of the *PPARγ* gene. This genotype revealed an unexpected twist to the role of PPARγ in insulin sensitivity (Kubota N, et al. H, Yamauchi T, et al. Mol. Cell 1999; 4:597-609). *PPARγ* +/− mice were partially resistant to weight gain when placed on a high-fat diet that caused adipocyte hypertrophy and obesity in their wild-type littermates. *PPARγ* +/− mice were also protected from the insulin resistance that accompanied weight gain in the wild-type animals. Therefore, the *PPARγ* +/− phenotype may be pharmacologically induced in the wild-type by treatment with a PPARγ partial agonist.

Thus improved methods are needed to treat an inflammatory diseases, said methods consisting of administering a pharmaceutically acceptable ligand selected from the group consisting of: 1) a PPARγ agonist which cross-activates PPARα (a PPARα/PPARγ dual agonist), 2) a PPARγ agonist which cross-activates PPARδ (a PPARγ/PPARδ dual agonist), 3) a PPARγ partial agonist, 4) a PPARγ/RXR agonist, effective to reverse, slow, stop, or prevent the pathological inflammatory or degenerative process. The present invention remedies such needs.

In other aspects, the present invention relates to a pharmaceutical composition comprising a compound of the present invention, or derivative thereof, or a pharmaceutically acceptable salt or solvate thereof; and a pharmaceutically acceptable carrier.

In another aspect, the present invention relates to a method of treating a PPAR-mediated inflammatory disease, comprising administering a therapeutically effective amount of a compound of the present invention or mixtures thereof to an individual suffering from a PPAR-mediated inflammatory disease.

Glossary

The term "peroxisome proliferator activating receptor-γ" or "PPARγ" refers to either the γ1, γ2 or γ3 isotypes or a combination of all isotypes of PPARγ. PPARs are nuclear receptors which naturally bind to fatty acids and which have been implicated in adipocyte differentiation (see, Perlmann *et al.*, *Cell*, **90**:391-397 (1997)).

The term "EC50" refers to the concentration of a compound required to activate 50% of the receptors that bind the compound present in a sample or a subject. Thus, in the present invention, the EC50 of a PPARγ modifier is the concentration of the modifier that activates 50% of the PPARγ present in the sample or organism. The term "activate" has its ordinary meaning, *i.e.*, cause to function or act.

The term "agonist" applies to a compound (ligand) that specifically binds and activates its target (cognate) receptor. For example, a PPARγ agonist specifically binds and activates all aspects of the PPARγ isoform. Thus, a PPARγ agonist ("full" agonist) specifically binds PPARγ and activates downstream expression of a specific pattern of genes.

Each isoform (e.g. PPARγ, PPARα or PPARδ) have unique and specific molecular requirements for the binding of their respective ligand, which may result in activation or partial activation of the respective receptor. These specific requirements of ligand-receptor specificity, affinity, activation and partial activation are apparent to those skilled in the art.

The term "partial agonist" applies to a compound (ligand) that specifically binds and partially activates its target (cognate) receptor. Partial activation refers to differential activation or incomplete activation, resulting in a *different* downstream gene expression pattern, compared to pattern triggered by an full agonist. Not wishing to be limited by example, the compound GW0072 is a PPARγ partial agonist which binds to PPARγ with high affinity, but only partially activates the receptor. "Full" activation of PPARγ (e.g. by exposure to rosiglitazone, a PPARγ agonist) promotes adipogenesis and differentiation of pre-adipocytes into mature adipocytes (terminal differentiation) and produces insulin-sensitization in vivo, whereas partial activation of PPARγ by exposure to GW0072 inhibits adipogenesis and differentiation of adipocytes without affecting the compound's insulin-sensitizing effects, in vivo. The chemical and molecular differences between a full agonist and a partial agonist, as the terms apply to PPARs, are apparent to those skilled in the art.

The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and animals, each unit containing a predetermined quantity of

active material calculated to produce the desired pharmaceutical effect in association with the required pharmaceutical diluent, carrier or vehicle. The specifications for the unit dosage forms of this invention are dictated by and dependent on (a) the unique characteristics of the active material and the particular effect to be achieved and (b) the limitations inherent in the art of compounding such an active material for use in humans and animals. Examples of unit dosage forms are tablets, capsules, pills, powder packets, wafers, suppositories, granules, cachets, teaspoonfuls, tablespoonfuls, dropperfuls, ampoules, vials, aerosols with metered discharges, segregated multiples of any of the foregoing, and other forms as herein described.

The terms "cancer, neoplasm or malignancy" include primary and metastatic disease. So, for example, cervical cancer includes the neoplasm at the primary site (cervix) and metastatic cervical cancer, regardless of site of metastasis, such as skeleton, brain, *etc.*

The term "inflammatory disease" includes diseases (treatable or preventable with compounds described in this invention) including, but not limited to,

a. T-lymphocyte activation and other T-lymphocyte-related disorders.

b. diseases involving the pathological production of inflammatory cytokines (e.g. TNF-α, interleukin (IL)-1β, IL-2, IL-6)

c. activation of nuclear factors that promote transcription of genes encoding inflammatory cytokines. Examples of these nuclear transcription factors include but are not restricted to: nuclear factor-κB (NF-κB), activated protein-1 (AP-1), nuclear factor of activated T cells (NFAT).

The term "diabetes," unless stated or qualified otherwise, refers to all variant forms of diabetes mellitus (DM), including type 1 DM, type 2 DM, gestational diabetes, etc.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a pharmaceutical composition comprising a PPARγ agonist which cross-activates PPARα, a PPARγ agonist which cross-activates PPARδ, or a PPARγ agonist which cross-activates both PPARα and PPARδ, or a PPARγ partial agonist, or a PPARγ/RXR agonist, derivatives thereof, their pharmaceutically acceptable salts or solvates thereof, compounded with a pharmaceutically acceptable carrier.

In certain aspects, the compounds, composition and methods of the present invention can be used to treat diseases involving tissues that express PPARγ, PPARα and PPARδ, and more particularly, can be used for treating inflammatory, proliferative, degenerative diseases

of multiple organs and tissues, and diseases involving pathological angiogenesis and neovascularization. Advantageously, the compounds can be used for treatment of diseases, tissues and organs regardless of etiological agent. For example, the treatment of corneal injury or ulceration caused by unrelated etiological agents is possible; these include, but are not limited to: 1) foreign body (e.g. contact lens), infectious agent (e.g. candida albicans, chlamydia trachomatis, cytomegalovirus or human immunodeficiency virus), physical agent (e.g. UV radiation), chemical agent (e.g. acids, caustic solvents) chronic systemic disease (e.g. autoimmune or collagen vascular diseases). Methods of the present invention for treating these inflammatory diseases comprise the administration of an effective amount of any natural or synthetic substance that modifies the activity of PPARγ, PPARα, PPARδ, or PPARγ/RXR.

In one embodiment, the methods of treatment are practiced by administering to a human in need thereof a dose of a compound (or pharmaceutically acceptable salts and solvates thereof in acceptable pharmaceutical excipients) that modifies the activity of PPARγ. The terms "modify and modulate" are defined to include its usually accepted meaning and includes treating a human subject prophylactically to alter inflammation, apoptosis, proliferation, angiogenesis, neovascularization, immune dysfunction, and expression of oncogenes and other genes controlling cell metabolism. The present method includes both medical therapeutic and/or prophylactic treatment, as necessary.

The compounds and methods described herein have clinical utility in the treatment of dermatological diseases (Table I), psychiatric disorders (Table II), neurodegenerative diseases (Table III), diseases associated with allograft transplantation (Table IV), inflammatory or degenerative diseases in multiple organ systems (Table V), neoplastic diseases (Table VIa, Table VIb), diseases caused by naked or coated DNA and RNA viruses (Table VII), diseases associated with human immunodeficiency virus (HIV) infection (Table VIII), inflammatory, proliferative and degenerative diseases of the eye (Tables IXa, IXb, IXc, IXd, IXe), and conditions associated with injury and age-related dysregulations (Table X).

In certain aspects, the methods of the present invention are practiced by administering to a mammal a dose of a compound, or a pharmaceutically acceptable salt, ester, solvate or tautomer thereof, a therapeutic amount that activates PPARγ and/or PPARα. The specific diseases and associated disorders that can be treated with the compounds are listed in Tables I through X. Using a method of the invention, therapeutic compounds are typically

administered to human patients topically to the skin or mucous membranes, by extra-ocular application, intraocularly (by chemical delivery system or invasive device), or systemically (e.g. sublingually, by suppository, by oral ingestion, intradermally, by inhalation, intramuscularly, intra-articularly, intravenously, or other parenteral route). Parenteral administration by a particular route is used in appropriate circumstances apparent to the practitioner. Oral administration is the preferred route for chronic diseases. Topical administration is the preferred route for dermatological diseases. Extra-ocular application is the preferred route for ocular diseases involving the anterior segment of the eye, or chronic diseases. Preferably, the compositions are administered in unit dosage forms suitable for single administration of precise dosage amounts.

To prepare a topical formulation for the treatment of ophthalmological or dermatological or other disorders described herein, a therapeutically effective concentration of the compound is placed in a dermatological vehicle as is known in the art. The amount of the therapeutic compound to be administered and the compound's concentration in the topical formulations depend upon the vehicle, delivery system or device selected, the clinical condition of the patient, the side effects and the stability of the compound in the formulation. Thus, the physician employs the appropriate preparation containing the appropriate concentration of the therapeutic compound and selects the amount of formulation administered, depending upon clinical experience with the patient in question or with similar patients.

The therapeutic compound is optionally administered topically by the use of a transdermal therapeutic system (see, Barry, Dermatological Formulations, p. 181 (1983) and literature cited therein). While such topical delivery systems have been designed largely for transdermal administration of low molecular weight drugs, by definition they are capable of percutaneous delivery. They can be readily adapted to administration of the therapeutic compounds of the invention by appropriate selection of the rate-controlling microporous membrane.

For ophthalmic applications the therapeutic compound is formulated into solutions, suspensions, and ointments appropriate for use in the eye. The concentrations are usually as discussed above for topico-local preparations. For ophthalmic formulations, see Mitra (ed.), Ophthalmic Drug Delivery Systems, Marcel Dekker, Inc., New York, N.Y. (1993) and also Havener, W. H., Ophthalmic Pharmacology, C.V. Mosby Co., St. Louis (1983).

The concentration of the therapeutic compound used depends on the mode of delivery. For topical ophthalmic and extraocular formulations, the concentration of the therapeutic compound is in the range of about 0.01% weight/weight (w/w) to about 10% w/w. Typically, the concentration of the therapeutic compound for this mode of delivery is in the range of about 0.025% w/w to about 2.5% w/w. Solid dispersions of the therapeutic compound as well as solubilized preparations can be used. For intraocular formulations (chemical delivery or delivery by invasive device), the therapeutic compound is delivered at a concentration high enough to achieve a final concentration in the range of about 0.1 μmol/L to about 10 μmol/L within the target ophthalmic compartment (e.g. the posterior chamber for the treatment of retinal diseases). Typically, for this mode of delivery, the final concentration of the therapeutic compound is in the range of about 0.25 μmol/L to about 5 μmol/L. Solid dispersions of the therapeutic compound as well as solubilized preparations can be used. Thus, the precise concentration is subject to modest but not undue experimental manipulation well within the skill of the ordinary medical practitioner in order to optimize the therapeutic response. Suitable vehicles include oil-in-water or water-in-oil emulsions for preparation of ointments using mineral oils, petrolatum, lanolin, glycerin and the like as well as gels such as hydrogel. A preferred embodiment of the present invention involves administration of semi-solid or solid implants containing PPARγ agonists.

In certain other aspects, the methods of the present invention include the use of all existing synthetic and naturally occurring PPARγ agonists and those yet to be discovered. Preferred PPARγ agonists useful for the application of methods described herein include the novel compounds described in the following submitted patent applications: Pershadsingh HA, Avery MA. 1,2-Dithiolane Derivatives, US Patent Application No. 09/520,208), and/or other drugs, which may be in slow release form for topical or systemic delivery. This may be accomplished in a preferred embodiment by using instrumentation and techniques described in U.S. Patent No. 5,817,075 and U.S. Patent No. 5,868,728.

For oral administration, either solid or fluid unit dosage forms can be prepared. For preparing solid compositions such as tablets, the compound of interest is mixed into formulations with conventional ingredients such as talc, magnesium stearate, dicalcium phosphate, magnesium aluminum silicate, calcium sulfate, starch, lactose, acacia, methylcellulose, and functionally similar materials as pharmaceutical diluents or carriers. Capsules are prepared by mixing the compound of interest with an inert pharmaceutical

diluent and filling the mixture into a hard gelatin capsule of appropriate size. Soft gelatin capsules are prepared by machine encapsulation of a slurry of the compound of interest with an acceptable vegetable oil, light liquid petrolatum or other inert oil. Fluid unit dosage forms for oral administration such as syrups, elixirs and suspensions can be prepared. The water soluble forms can be dissolved in an aqueous vehicle together with sugar, aromatic flavoring agents and preservatives to form a syrup. An elixir is prepared by using a hydroalcoholic (e.g., ethanol) vehicle with suitable sweeteners such as sugar and saccharin, together with an aromatic flavoring agent. Suspensions can be prepared with an aqueous vehicle with the aid of a suspending agent such as acacia, tragacanth, methylcellulose and the like.

Appropriate formulations for parenteral use are apparent to the practitioner of ordinary skill. Usually, the therapeutic compound is prepared in an aqueous solution (discussed below) in a concentration of from about 1 to about 100 mg/ml. More typically, the concentration is from about 10 to 60 mg/ml or about 20 mg/ml. Concentrations below 1 mg/ml may be necessary in some cases depending on the solubility and potency of the compound selected for use. The formulation, which is sterile, is suitable for various topical or parenteral routes including sublingual, by suppository (e.g. per-rectum or vaginal application), oral, intravascular, intradermal, by inhalation, intramuscular, intra-articular, intravenous, or other parenteral route.

In addition to the therapeutic compound, the compositions may include, depending on the formulation and mode of delivery desired, pharmaceutically-acceptable, non-toxic carriers or diluents, which include vehicles commonly used to form pharmaceutical compositions for animal or human administration. The diluent is selected so as not to unduly affect the biological activity of the combination. Examples of such diluents which are especially useful for injectable formulations are water, the various saline, organic or inorganic salt solutions, Ringer's solution, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation may include additives such as other carriers; adjuvants; or nontoxic, nontherapeutic, nonimmunogenic stabilizers and the like.

Furthermore, excipients can be included in the formulation. Examples include cosolvents, surfactants, oils, humectants, emollients, preservatives, stabilizers and antioxidants. Any pharmacologically acceptable buffer may be used, e.g., tris or phosphate buffers. Effective amounts of diluents, additives and excipients are those amounts which are effective to obtain a pharmaceutically acceptable formulation in terms of solubility, biological

activity, etc.

In certain preferred aspects, a composition of the invention includes a therapeutic compound which may be formulated with conventional, pharmaceutically acceptable, vehicles for topical, oral or parenteral administration. Formulations may also include small amounts of adjuvants such as buffers and preservatives to maintain isotonicity, physiological and pH stability. Means of preparation, formulation and administration are known to those of skill. See generally Remington's Pharmaceutical Science 15th ed., Mack Publishing Co., Easton, Pa. (1980).

Slow Release Delivery

Slow or extended-release delivery systems, including any of a number of biopolymers (biological-based systems), systems employing liposomes, colloids, resins, and other polymeric delivery systems or compartmentalized reservoirs, can be utilized with the compositions described herein to provide a continuous or long term source of therapeutic compound. Such slow release systems are applicable to formulations for delivery via topical, intraocular, oral, and parenteral routes.

Delivery by Invasive Device

As mentioned above, delivery intravascularly, intra-articularly, intramuscularly, intra-articularly, intradermally, or other parenteral route can be accomplished by injection, cannula or other invasive device designed to introduce precisely metered amounts of a desired formulation to a particular compartment or tissue. For example, delivery to certain areas within the eye, in situ, can be accomplished by injection, cannula or other invasive device designed to introduce precisely metered amounts directly or contained in a reservoir for slow release in situ, of a desired formulation to a particular compartment or tissue within the eye (e.g. anterior or posterior chamber, uvea or retina). Preferably, a solid or semisolid implant can be delivered subretinally using the instrumentation and methods described in U.S. Patent Nos. 5,817,075 and 5,868,728.

Routes of Administration

In certain aspects, therapeutic agents of the present invention are delivered or administered topically for treating disorders involving the eye that are listed in Tables I through X. Oral administration is preferred for disorders in Tables I through X that cannot be treated effectively by topical therapy. Additionally, the agents can be delivered parenterally, especially for treatment of retinitis and degenerative retinal diseases, and for other conditions

in Tables I through X, that do not respond to oral or topical therapy, or for conditions where oral or topical therapy is not feasible. Parenteral therapy is typically oral, intraocular, transcutaneous, intradermal, intrathecal, intramuscular, intra-articular, by inhalation, intravascular, sublingual, by suppository (e.g. per-rectum or vaginal application), by inhalation, or other parenteral route.

A preferred way to practice the invention for dermatological or ophthalmic disorders in Tables I through XI to which this method is applicable, is to apply the compound of interest, in a cream, lotion, ointment, or oil based carrier, directly to the lesion. Typically, the concentration of therapeutic compound in a cream, lotion, or oil is 0.1 to 2.5%. In general, the preferred route of administration is oral, topical, intraocular or parenteral. Topical administration is preferred in treatment of lesions of the skin as in psoriasis, external eye as in conjunctivitis, keratitis, scleritis, squamous cell carcinoma, corneal erosion, dry eye syndrome, and anterior compartment of the eye as in glaucoma, uveitis and other diseases of the uveal tract, where such direct application is practical and clinically indicated.

Oral administration is a preferred alternative for treatment of other lesions discussed in Tables I through XI, where direct topical application is not useful as in the treatment of chronic or acute systemic diseases, and diseases of the posterior segment of the eye, as in retinitis and other retinal degenerative diseases. Intravascular (intravenous being the preferred route) administration may be necessary in disorders that cannot be effectively treated by topical or oral administration.

Intraocular, transcutaneous, intradermal, intrathecal, intramuscular, intra-articular injections or other invasive technique are preferred alternative in cases where the practitioner wishes to treat one or a few specific areas or lesions depending on their location within the eye. Usually, the compound is delivered in an aqueous solution. Additionally, the therapeutic compounds are injected directly into lesions (intra-lesion administration) in appropriate cases. Intradermal administration is an alternative for extraocular lesions. Intra-lesional and intradermal injections are alternative routes of application for certain lesions, e.g. extraocular neoplastic or hyperplastic lesions such as squamous cell carcinoma and condyloma, respectively. Inhalation therapy is preferred for pulmonary diseases, sublingual and intra-rectal suppository is preferred for rapid delivery or in clinical situations where delivery via the oral or intravascular route is inconvenient or problematic. Application via vaginal topical formulation or via suppository formulation is preferred for diseases localized

to the vagina or other segment of the urogenital tract.

For pulmonary applications, a chemical delivery system for drug targeting to lung tissue using the 1,2-dithiolane-3-pentyl moiety of lipoic acid as the "targetor moiety". Therefore a preferred therapeutic compound is the 1,2-dithiolane-3-pentyl ester derivative of any PPARγ or PPARα agonist and is formulated into solutions, suspensions, aerosols and particulate dispersions appropriate for application to the pulmonary system. The therapeutic agent may be inhaled via nebulizer, inhalation capsules, inhalation aerosol, nasal solution, intra-tracheal as a solution via syringe, or endotracheally tube as an aerosol or via as a nebulizer solution. In vitro kinetic and in-vivo pharmacokinetic studies have shown that the 1,2-dithiolane-3-pentyl ester moiety provides an effective pulmonary delivery system which, in a sufficiently stable in buffer and biological media, is hydrolyzed rapidly into the respective active parent drugs, with significantly enhanced delivery and retention of the active compound to lung tissue.

Dosage and Schedules

An effective quantity of the compound of interest is employed in treatment. The dosage of compounds used in accordance with the invention varies depending on the compound and the condition being treated. For example, the age, weight, and clinical condition of the recipient patient; and the experience and judgment of the clinician or practitioner administering the therapy are among the factors affecting the selected dosage. Other factors include: the route of administration, the patient, the patient's medical history, the severity of the disease process, and the potency of the particular compound. The dose should be sufficient to ameliorate symptoms or signs of the disease treated without producing unacceptable toxicity to the patient. In general, an effective amount of the compound is that which provides either subjective relief of symptoms or an objectively identifiable improvement as noted by the clinician or other qualified observer.

Broadly, for a PPAR ligand (PPARα, PPARγ or PPARδ), the oral dose is determined from the following formula:

oral dose (in mg)= (k1)(EC50)(k2) (LBW)(MW);

wherein k1 is a dimensionless constant of 5 to 100;

EC50 is the concentration (amount) of compound required to activate or bind to 50% of the PPAR ligand in the sample or patient and is in mole/L units;

k2 is the fractional water content of the lean body weight (LBW) of the patient = 0.72

L/kg, (see, Geigy Scientific Tables, Vol. 1, Lentner (ed.), p217, Giba-Geigy Ltd., Basle, Switzerland (1981); and

MW is the molecular weight of the compound in g/mole.

For example, troglitazone is a compound encompassed by the methods of this invention. A man with diagnosis of early stage prostate cancer in situ has a lean body weight (LBW) of 70 kg. If k1 = 10; the EC50 for troglitazone = 2.4 x 10 -6 mol/L and the molecular weight of troglitazone = 442 g/mol, then the oral dose in milligrams= (10)(2.4 x 10 -6 mol/L)(0.72 L/kg x 70 kg) (442 g/mol) or 535 mg. Similarly, an effective dose of rosiglitazone in milligrams for an average man is (10) (0.06 x 10-6 mol/L) (0.72L/kg x 70kg) (304 g/mole) or 9.2 mg.

Typically, the dosage per day of a thiazolidinedione of this invention will depend on the affinity of the thiazolidinedione for PPARγ. The dosages of compounds with high affinity, e.g., rosiglitazone, will fall between about 0.5 mg to about 100 mg, of compounds of intermediate affinity will fall from about 10 mg to about 500 g and compounds with low affinity, e.g., troglitazone, will fall from about 100 mg to about 5 g.

An oral dosing schedule is typically, a single dose once a day. However, more than one dose can be given per day. Because of the lower incidence of undesirable side effects, the compounds of this invention can be given until improvement in the inflammatory process or disease involving neovascularization is observed.

Because the compounds of this invention are to some degree fat-soluble, in a preferred embodiment, the compounds are administered with food. The fats in food provide a lipid micellular phase in which the PPARγ and/or PPARα modifiers of this invention can solubilize and be more effectively absorbed.

A dosage range for local treatment is about 0.1% to about 10% (weight/volume) in a suitable solvent applied that permits release of the compound into the prostate tissue. One of skill will realize that the dosage for local treatment will vary depending on the compound used. For example, the thiazolidinediones of this invention have different affinity for PPARα and/or PPARγ, e.g., pioglitazone has a higher affinity for PPARγ than troglitazone. Typically, the greater the affinity, the more effective the compound, and the lower the dosage that is an effective amount. Therefore, a lower concentration of pioglitazone in a unit dosage form comprises an effective amount.

Typically, the local dosage is administered at least once a day until a therapeutic result

is achieved. The dosage can be administered twice a day, but more or less frequent dosing can be recommended by the clinician. Once a therapeutic result is achieved, the compound can be tapered or discontinued. Occasionally, side effects warrant discontinuation of therapy.

An effective quantity of the compound of interest is employed in treatment. The dosage of compounds used in accordance with the invention varies depending on the compound and the condition being treated. The age, lean body weight, total weight, body surface area, and clinical condition of the recipient patient; and the experience and judgment of the clinician or practitioner administering the therapy are among the factors affecting the selected dosage. Other factors include the route of administration the patient, the patient's medical history, the severity of the disease process, and the potency of the particular compound. The dose should be sufficient to ameliorate symptoms or signs of the disease treated without producing unacceptable toxicity to the patient.

Broadly, an oral dosing schedule is from about 0.1 mg to about 1000 mg once or twice a day depending on the binding affinity of the compound for PPARγ. For example, typical oral FDA-approved doses of the thiazolidinediones, rosiglitazone and pioglitazone for the treatment of type 2 diabetes, are 4 to 8 mg, and 15 to 45 mg daily, respectively.

Using troglitazone as the prototype agent for the purpose of this invention, a convenient oral dose for an adult patient is 300 to 600 mg once a day. A dosage range for topical treatment is about 0.5% to about 5% (weight/volume) in a gel, cream or ointment, applied twice a day. A usual dose for intramuscular or intraocular injection is 1 to 10 mg, depending on the compartment of the eye to be treated and on the lean body mass of the patient. A typical dosage for intra-dermal administration is about 5 to 50 mg per injection per site. A typical dosage for intravenous or intramuscular administration in an adult patient would be between 100 and 400 mg per day given in single or divided doses depending on the judgement of the practitioner.

Typically, the dosage is administered at least once a day until a therapeutic result is achieved. Preferably, the dosage is administered twice a day, but more or less frequent dosing can be recommended by the clinician. Once a therapeutic result is achieved, the drug can be tapered or discontinued. Occasionally, side effects warrant discontinuation of therapy. In general, an effective amount of the compound is that which provides either subjective relief of symptoms or an objectively identifiable improvement as noted by the clinician or other qualified observer.

The compounds in this invention can also be given orally in combination with natural or synthetic compounds that bind to or modify the activity of the vitamin D receptor or in combination with compounds that bind to or modify the activity of the retinoid X receptor to provide for a synergistic effect in the treatment or prevention of the disorders listed in Tables I through XI. Examples of such compounds that provide for synergistic effect when given in combination with the drugs encompassed by the current invention include vitamin D analogs, various retinoic acid derivatives, and other ligands for retinoid X receptors or retinoic acid receptors including but not limited to compounds such as LG100268, tazarotene, TTNPB, AGN 190121, adapalene or LGD1069 (Targretin).

Synergistic therapeutic effects can be achieved by oral or topical administration of the drugs encompassed in the current invention together with orally, topically or intravenously administered drugs that bind to and modify the activity of either the vitamin D receptor, the glucocorticoid receptor, the intracellular enzyme calcineurin, the retinoid X receptors, or the retinoic acid receptors. A preferred dosage range for administration of a retinoic acid derivative or retinoid would typically be from 0.1 to 100 mg per square-meter of body surface area, depending on the drug's ability to bind to or modify the activity of its cognate nuclear receptor, given in single or divided doses, orally or by continuous infusion, two or three times per day. For synergistic therapy, the preferred dosages and routes and frequency of administration of the vitamin D analogs or retinoid compounds can be similar to the dosages and routes and frequency of administration ordinarily recommended for these agents when given without PPARγ activators. Examples of effective retinoids are 9-cis-retinoic acid, 13-cis-retinoic acid, all-trans-retinoic acid (at-RA). Preferred retinoids for this purpose would include 13-cis-retinoic acid, tazarotene, or Targretin. A preferred dosage range for systemic administration of a vitamin D analog would typically be from 0.1 to 100 mg per square-meter of body surface area, depending on the drug's ability to bind to and or activate its cognate vitamin D receptor, given in single or divided doses, orally or by continuous infusion, two or three times per day. Examples of effective vitamin D analogs are 1,25-dihydroxy-vitamin D, calcipotriene and calcipotriol. The dosage range and routes and frequency of administration of PPARγ activators required to achieve synergistic effects when given with vitamin D or retinoid derivatives are the same as those described elsewhere in this disclosure. The preferred mode of administration of these drugs for synergistic therapeutic purposes would be orally although alternatively one can use topical or parenteral routes of administration. The

dosages and the modes and frequency of administration of the vitamin D or retinoid related compounds for synergistic topical therapy would be similar to those ordinarily recommended for these agents when given without PPARγ activators. The dosage range and the modes and frequency required for topical administration of the flavonoid thiazolidine derivatives given in combination with vitamin D or retinoid related compounds are the same as those described elsewhere in this disclosure.

Synergistic therapeutic effects can be achieved by oral or topical administration of the drugs encompassed in the current invention together with orally, topically or intravenously administered natural or synthetic antioxidants. These include vitamin D derivatives (e.g. calcipotriene and calcitriol), antioxidant vitamins such as ascorbic acid and the tocopherols (e.g. vitamin E, vitamin E succinate), carotenes and carotenoids (e.g. β-carotene), α-lipoic acid, probucols, flavones, isoflavones and flavonols (e.g. quercetin, genistein, catechin, apigenin, lutein, luteolin), glutathione and its derivatives (e.g. N-acetylcysteine and dithiothreitol), and phytoestrogens and phenolic anthocyanidin and procyanidin derivatives (e.g. resveratrol, cyanidin, cinnamic acid).

The compounds of the instant invention are further useful to suppress the mediators of neurogenic inflammation (e.g. substance P or the tachykinins), and may be used in the treatment of rheumatoid arthritis; psoriasis; topical inflammation such as is associated with sunburn, eczema, or other sources of itching; and allergies, including asthma. The compounds of this invention can also function as neuromodulators in the CNS, with useful applications in the treatment of Alzheimer's disease and other forms of dementia, pain (as a spinal analgesic), allodynia, and headache. Furthermore, in disorders involving myocardial fibrosis, myocardial ischemia, pathological conditions secondary to the autoimmune response to allograft transplantation, the splanchnic blood flow, including hepatic fibrosis, cirrhosis and oesophagal varices, the compounds of the invention can provide cytoprotection.

This application claims the use of compounds described in US Provisional Application No. 60/283774 filed April 12, 2001 that act as activators of PPARγ as full or partial agonists, as antiinflammatory agents for the purpose of this invention.

SOURCE INFORMATION ON COMPOUNDS LISTED IN THIS APPLICATION

Compound	**Reference**
MCC 555	1,2

3-substituted benzyl)thiazolidine-2,4-diones, e.g. KRP 297	1,3
Indole-based PPARgamma agonists, e.g. BRL 48482	4
5-aryl-2,4-thiazolidinedione	5
isoxazolinedione derivatives, e.g. JTT-501	1,6,7
alpha-methoxy-beta-phenylpropanoic acids, e.g. SB 236636	1, 8,9
N-(2-Benzoylphenyl)-L-tyrosine derivative, e.g. GI262570, GW7845	1,10,11,12
Phenylacetic acid derivatives, e.g. L-764486, L-764406	13,14
LG100268, LGD1069, LG100268, LGD100324, LG100754	1,15
NNC 61-0029 (DRF-2725)	16
AZ 242	17

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EXAMPLES OF HOW TO PRACTICE THE INVENTION

Example 1: Biological assay to screen compounds of the present invention that are activate PPARγ, PPARα or PPARδ

Compounds of the present invention are activators of PPARγ, PPARα or PPARδ, or any combination thereof. As described hereinbelow, a transient cotransfection assay can be used to screen for PPAR activity. In this assay, chimeras are constructed that fuse the ligand binding domains of each PPAR subtype to the DNA binding domain of the yeast transcription factor GAL4. Expression plasmids for the GAL4-PPAR chimeras are then transfected into cells with a reporter construct. This general assay system identifies compounds which are activators of PPARγ (full and/or partial agonists) and/or PPARα and/or PPARδ (see, Lehmann *et al., J. Biol. Chem.* **270**:12953-12956 (1995) and Murakami, K *et al., Biochem. Biophys. Res. Commun.* **260**: 609-613 (1999) for specific protocols).

Example 2: Method for Screening for Compounds that Modify the Activity of PPARγ and PPARα based on Inhibition of NF-κB activation

The (3-substituted benzyl)thiazolidine-2,4-dione dithiolane derivatives bind and activate both PPARα and PPARγ. These compounds are tested for the ability to inhibit activity of NF-κB. Isolated human T lymphocytes or a mammalian cell line such as a Jurkat T cell line which expresses PPARγ is stimulated with a concentration of one or a combination of: phytohemagglutinin/phorbol 12-myristate 13-acetate (PHA/PMA), TNF-α, interferon-γ or some other factor that activates NF-κB. Activation of NF-κB is determined by electrophoretic mobility shift assay similar to that described by Rossi *et al.* Preincubation of the same cells with 5 μM of the test compound 2 hours prior to addition of an activator of NF-κB inhibits the activation of NF-κB otherwise observed in the absence of a PPAR ligand

Example 4: Method for Screening for Compounds that Modify the Activity of PPARγ and PPARα based on Inhibition of NFAT activation

Isolated human T lymphocytes or a mammalian cell line such as a Jurkat T cell line which expresses PPARγ is stimulated with a concentration of one or a combination of PHA/PMA, TNF-α, interferon-γ or some other factor that activates the nuclear factor activator of T cells (NFAT). Transcriptional activation of NFAT is determined by electrophoretic mobility shift assay similar to that described by Yang *et al.* Preincubation of the same cells with 5 μM of the test compound for 2 hr prior to addition of an activator of NFAT inhibits the activation of NFAT otherwise observed in the absence of said compound.

Example 5: Method for Screening for Compounds that Modify the Activity of PPARγ and PPARα based on Inhibition of IL-2 production

Isolated human T lymphocytes or a mammalian cell line such as a Jurkat T cell line which expresses PPARγ is stimulated with a concentration of one or a combination of PHA/PMA, TNF-α, interferon-γ or some other factor that activates induction of IL-2 gene expression. Production of IL-2 is determined by measuring the concentration of IL-2 in the supernatant from cells using Endogen kits (Wolburn), as described by Yang *et al.* Preincubation of the same cells with 5 micromolar of the test compound for 12 hours prior to addition of an activator of IL-2 production inhibits the activation of IL-2 production otherwise observed in the absence of said compound.

Example 6: Methods of determining the anti-apoptotic effect of PPARγ ligands in PPARα or PPARγ-expressing cells

Apoptosis (cell death) is induced by adding TNF-α (Genzyme, USA) to peripheral T lymphocytes or Jurkat cells in culture. The inhibitory activity of a test compound to this apoptosis is determined by using dexamethasone as the standard, a compound known to have apoptosis inhibitory activity. An aliquot of RPMI-1640 culture medium (containing 10 weight % of fetal bovine serum) is added to each well of a 96-well microplate. Then, a test solution of the candidate compound in dimethylsulfoxide is added to the culture medium to give the desired final concentration (0.1 to 10 μM). Subsequently, TNF-α (40 ng/ml, final concentration) is added to the culture medium, and cells incubated for 72 hours at 37 degree C in the presence of 5% carbon dioxide in air. After cultivation, the culture medium is

removed from wells by aspiration, and 50 µl of a 5%(w/v) crystal violet/70%(v/v) methanol solution added to each well to stain living cells. The wells are washed, dried and apoptosis inhibitory activity of the test compound obtained by determining the optical density using an absorption spectrometer (Microplate Reader Model 450, produced by Bio-Rad) at $\lambda = 570$ nm. Dexamethasone standard was compared to the test compound, final concentration 1 µM.

Example 7: Treatment of an Intraocular Disease Selected from the Following diseases: Glaucoma, Retinopathy, Optic Neuritis, Retinitis, Macular Degeneration, by Oral Administration of a PPARα/PPARγ agonist, e.g. KRP 297 or MCC 555

Early disease: A patient having early ophthalmic manifestations of an optic neuritis (e.g. optic neuritis associated with multiple sclerosis), a retinitis (e.g. retinitis pigmentosa), or a retinopathy (e.g. hypertensive, diabetic or glaucomatous retinopathy), or a maculopathy (e.g. macular degeneration), is selected for therapy. The patient weighs 70 kilograms, and if female of child-bearing capacity, is given a pregnancy test to confirm that she is not pregnant. Provided that the patient is not pregnant and does not plan to become pregnant during treatment, KRP 297 or MCC 555 is administered orally in a dosage of about 25 mg twice daily with a fat-containing meal. The patient is evaluated by an ophthalmologist experienced in the ophthalmic manifestations of retinal diseases at monthly intervals for 3 months. Regression of the disease or improvement in his clinical status is evaluated by monitoring the visual fields, color vision and visual acuity. If regression is not evident or minimal, the dose is increased to 50 mg twice daily. Additionally, a complete blood count, including white cell count and differential, a platelet count, and liver function tests (such as levels of alkaline phosphatase, lactate dehydrogenase, and aminotransferases) are checked prior to treatment and monthly thereafter. The dosage is tapered to maintenance, 25 mg daily.

Late disease: A similar patient with late ophthalmic manifestations one of the diseases described, is selected for therapy. The approach is the same as for the foregoing patient, except that the starting dose is 50 mg twice daily. After 12 months, the dose may be decreased to a maintenance dose of 25 mg once or twice daily.

Example 8: A Clinical Trial, Synergistic (Adjunctive) Therapy for Preventing Acute and Chronic Allograft Rejection

The balance between acute rejection and infection after transplantation continues to be of significant clinical concern, especially during the early post-transplantation period. Acute

rejection is a significant risk factor for chronic rejection, and chronic rejection is an important cause of late graft loss. Monoclonal antibodies that selectively block the interleukin-2 receptors on activated T-helper cells are used for immunoprophylaxis or anti-lymphocyte globulins for induction therapy to provide reduced dosing of cyclosporine A throughout the early post-transplantation course. In the context of the present invention, a PPARγ agonist is effective adjunctive therapy for preventing acute and chronic allograft rejection. The PPARγ agonist is useful for providing reduced dosing of immunosuppressive therapy, including cyclosporine A, tacrolimus, azathioprine, mycophenolate or other related therapy to preventing allograft rejection throughout both early and late phases post-transplantation. The PPARγ agonist is used with one or more anti-rejection drug, or in combination with a RXR agonist, or a PPARγ/RXR agonist, and/or a vitamin D receptor agonist, and/or a glucocorticoid receptor agonist, and/or an estrogen receptor agonist, and/or an androgen receptor agonist. To achieve a synergistic effect, the treatment can be modified to include combination therapy with a thiazolidinedione (PPARγ ligand) or rexinoid (e.g. LG100754, a PPARγ/RXR heterodimer ligand) and another immunosuppressive compound traditionally used for preventing allograft rejection. Examples of such compounds that provide for synergistic effect when given in combination with the drugs encompassed by the current invention include ligands for the glucocorticoid nuclear receptor ligand (e.g. prednisone), inhibitors of purine synthesis (e.g. azathioprine and mycophenolate), and inhibitors of the calcineurin-dependent cytokine synthesis in activated lymphocytes (e.g. cyclosporine, tacrolimus, sirolimus). One or a combination of these compounds are employed (at dosages described above in *Dosage and Schedules*) in clinical trials at doses sufficient to prevent or treat allograft rejection. The PPARγ/PPARα dual agonist is selected from the group consisting of: KRP 297, MCC 555, JTT-501, AZ 242, NNC 61-0029 (DRF-2725) up to 50 mg daily (15 to 30 mg preferred). The PPAR ligand may also be a PPARγ partial agonist, and is administered alone or with another agent to provide a synergistic clinical effect. Examples are:

a. A PPARγ/PPARα agonist is administered in combination with prednisone at an FDA-approved dose.

b. A PPARγ/PPARα agonist is administered in combination with prednisone ***and*** cyclosporine A or tacrolimus at an FDA-approved dose, or sirolimus at a dose used in clinical trials.

c. A PPARγ/PPARα agonist is administered in combination with prednisone ***and*** cyclosporine A or tacrolimus or sirolimus, ***and*** azathioprine or mycophenolate.

d. A PPARγ partial agonist such as GW0072, is administered in combination with one or more FDA-approved immunosuppressive transplant rejection therapeutic compound, as described in examples a, b and c above.

e. A rexinoid PPARγ/RXR heterodimer ligand (e.g. LG100754) is administered in combination with one or more FDA-approved immunosuppressive transplant rejection therapeutic compound at approved dosages as described in examples a, b and c above.

Example 9: Treatment of Chronic Recalcitrant Multiple Sclerosis by Oral Administration of Pioglitazone - A Clinical Trial

The following is an example for treating chronic recalcitrant multiple sclerosis (MS) with the PPARγ agonist, pioglitazone. PPARγ/PPARα dual agonists selected from the group consisting of: KRP 297, MCC 555, JTT-501, AZ 242, NNC 61-0029 (DRF-2725) up to 50 mg daily (15 to 30 mg preferred), may also be employed in an analogous fashion.

Early disease: The patient presents acutely with the neurological manifestations of multiple sclerosis, and the diagnosis is confirmed by clinical laboratory and pathological diagnostic tests. The patient is evaluated by a neurologist experienced in the clinical and laboratory manifestations of multiple sclerosis lesions. The patient weighs 70 kilograms, and if female of child-bearing capacity, is given a pregnancy test to confirm that she is not pregnant. Provided that the patient is not pregnant and does not plan to become pregnant during treatment, a compound known to activate PPARγ, namely, the thiazolidinedione, pioglitazone is administered orally in a dosage of 15 milligrams daily during the acute episode, and is titrated up to 30 mg daily then 45 mg daily at weekly intervals. Regression of the disease or improvement in his clinical status is evaluated by monitoring improvement in motor deficits. Reduction of the systemic inflammation associated with the disease is assessed by performing bi-monthly measurements of high sensitivity-C-reactive protein (hs-CRP). A reduction in the hs-CRP by 50% within 3 months of initiating therapy is considered to be a positive response to the therapy. Additionally, a complete blood count, white cell count and differential, a platelet count, liver function tests (such as levels of alkaline phosphatase, lactate dehydrogenase, and aminotransferases), erythrocyte sedimentation rate and plasma interleukin-2 levels are checked prior to treatment and monthly thereafter. After 6 months

treatment, the dosage is tapered to a maintenance dose of 30 mg.

Late disease: A similar patient with chronic recalcitrant multiple sclerosis, having failed existing approved therapies such as interferon injections, and with late manifestations of the disease, such as weight loss, cachexia, rigidity, vision loss, or quadriplegia, is selected for therapy. The approach is the same as for the foregoing patient, except that the starting dose of 30 mg pioglitazone once daily for 3 months, and is increased to 45 mg thereafter. Regression of the disease or improvement in his clinical status is evaluated by monitoring improvement in motor deficits. A reduction in the hs-CRP by 50% within 3 months of initiating therapy is considered to be a positive response to the therapy.

Example 10: Treatment of a PPAR-Mediated Inflammatory, Proliferative or Degenerative Disease with a PPARγ/PPARα Dual Agonist or a PPARγ/PPARδ Dual Agonist.

The PPAR-mediated disease is selected from one of the following: a degenerative neurological disease (e.g. Alzheimer's disease), rheumatoid arthritis, atherosclerosis, depression, post-myocardial cardiomyopathy, congestive heart failure, hepatic fibrosis, a pulmonary disease (asthma or chronic obstructive pulmonary disease), a carcinogenic disease, or other inflammatory, proliferative, or degenerative disease (Horrocks LA, Yeo YK. Pharmacol Res 1999; 40:211-25; Youdim KA, Int J Dev Neurosci. 2000; 18:383-99; Martinez M, et al. Rev Neurol 1999; 28 Suppl 1:S59-64).

The PPARγ/PPARα dual agonist is selected from the group consisting of: MCC 555, JTT-501, AZ 242, NNC 61-0029 (DRF-2725) with a dose of up to 50 mg daily (15 to 30 mg preferred). The PPARγ/PPARδ dual agonist is selected from the group consisting of:GW9578, KRP 297, with a dose of up to 50 mg daily (15 to 30 mg preferred).

The patient presents acutely or chronically ill and the diagnosis is confirmed by clinical laboratory and pathological diagnostic tests. The patient is evaluated by a specialist experienced in the clinical and laboratory manifestations of the index disease. The patient weighs 70 kilograms, and if female of child-bearing capacity, is given a pregnancy test to confirm that she is not pregnant. Provided that the patient is not pregnant and does not plan to become pregnant during treatment, one of the compounds identified above is administered orally in a dosage of 10 mg daily, and is titrated up to 30 mg daily at weekly intervals. Regression of the disease or improvement in his clinical status is evaluated by monitoring standard clinical indicators. Additionally, a complete blood count, white cell count and

differential, a platelet count, liver function tests (such as levels of alkaline phosphatase, lactate dehydrogenase, and aminotransferases), erythrocyte sedimentation rate and plasma high sensitivity-C-reactive protein are checked prior to treatment and monthly thereafter. After 3 to 6 months treatment, the dosage is tapered to a maintenance dose of 10 or 20 mg daily. The patients response to therapy is monitored by laboratory markers of the respective disease, and inflammatory markers of systemic inflammation to monitor amelioration of the inflammatory response to assess clinical improvement.

Example 11: Combination Treatment of a PPAR-Mediated Inflammatory, Proliferative or Degenerative Disease with PPARγ Agonist or a Mixed PPARγ/PPARα Agonist (Co-Ligand) and an Estrogen Receptor Ligand - A Clinical Trial

The PPAR-mediated disease is selected from one of the following: a degenerative neurological (Alzheimer's disease) or uveo-retinal disease, arthritis, atherosclerosis, depression, cardiomyopathy, congestive heart failure, post-myocardial fibrosis, hepatic fibrosis, pulmonary disease (asthma, COPD), a carcinogenic disease, or other inflammatory, proliferative, or degenerative disease (Horrocks LA, Yeo YK. Pharmacol Res. 1999; 40:211-25; Youdim KA, Int J Dev Neurosci. 2000; 18:383-99; Martinez M, et al. Rev Neurol 1999; 28 Suppl 1:S59-64).

The PPARγ agonist or mixed PPARγ/PPARα agonist or co-ligand are 4-substituted or 3-substituted benzodithiolanyl derivatives (e.g. KRP 297), respectively described in this invention, administered at doses of 1 to 2 mg twice daily oral dose. Examples of other mixed PPARγ/PPARα co-ligands are KRP 297 (50 to 500 mg, daily oral dose. The estrogen receptor (ER) ligand is selected from: estradiol (0.5 to 10 mg, daily oral dose, 1.25 mg preferred), tamoxifen or 4-OH-tamoxifen (5 to 50 mg, daily oral dose, 15 mg preferred), clomifene, coumestrol, genistein (10 to 200 mg, daily oral dose, 50 mg preferred), or biochanin A, a genistein precursor (5 to 100 mg, daily oral dose, 20 mg preferred). These pharmacological compositions may be used to treat acute or chronic disease or may be used prophylactically to prevent the onset of the disease.

The patient presents acutely or chronically with the manifestations of Alzheimer's disease (a neuro-degenerative disease), glaucomatous retinopathy (a neuro-retinal degenerative disease), atherosclerosis (an inflammatory ischemic vascular disease), ulcerative colitis (an inflammatory bowel disease), hepatic fibrosis (a degenerative liver disease), or a

carcinogenic disease of the breast or prostate. The diagnosis is confirmed by clinical laboratory and pathological diagnostic tests. The patient is evaluated by a specialist experienced in the clinical and laboratory manifestations of the index disease. The patient weighs 70 kilograms, and if female of child-bearing capacity, is given a pregnancy test to confirm that she is not pregnant. Provided that the patient is not pregnant and does not plan to become pregnant during treatment, KRP 297 is administered orally in a dosage of 100 mg twice daily. Regression of the disease or improvement in his clinical status is evaluated by monitoring standard clinical indicators. Additionally, a complete blood count, white cell count and differential, a platelet count, liver function tests (such as levels of alkaline phosphatase, lactate dehydrogenase, and aminotransferases), erythrocyte sedimentation rate and plasma high sensitivity-C-reactive protein are checked prior to treatment and monthly thereafter. The patients response to therapy is additionally monitored by laboratory markers of the respective disease, and inflammatory markers of systemic inflammation to monitor amelioration of the inflammatory response to determine clinical improvement.

Example 12: Combination Treatment of a PPAR-Mediated Inflammatory, Proliferative Dermatological (Skin) Disease with PPARγ Agonist or a Mixed PPARγ/PPARα Agonist (Co-Ligand) and a Vitamin D Receptor Ligand - A Clinical Trial

The PPAR-mediated disease is an inflammatory, proliferative or degenerative skin disease such as psoriasis, keratitis, hidradenitis, ichthyosis, acne, rosacea, verrucae and other HPV infections, atopic dermatitis, allergic dermatitis, chemical (irritant) dermatitis, seborrheic dermatitis, solar dermatitis, acute and chronic eczema, seborrheic keratosis, senile keratosis, actinic keratosis, photo-induced keratosis, skin aging, thinning skin, dry skin, wrinkle formation, photo-induced skin aging, keloids, lichen planus.

The PPARγ agonist or mixed PPARγ/PPARα agonist or co-ligand are 4-substituted or 3-substituted benzodithiolanyl derivatives, respectively described in this invention, administered at doses consistent with the EC50 for PPARα and/or PPAR, and with the pharmacokinetic area under the curve (AUC), and is given as a once or twice daily oral dose, or in a pharmaceutical composition for topical administration, with active ingredient at a concentration ranging from 0.01 to 2.0%, 0.25% preferred. PPARγ-specific agonists are selected from the group consisting of: a thiazolidinedione given orally, e.g. rosiglitazone, 4 mg twice daily or pioglitazone, 45 mg once daily). Examples of mixed PPARγ/PPARα co-

ligands are KRP 297 (25 to 250 mg, daily oral dose). The vitamin D receptor (VDR) ligand is a natural or synthetic vitamin D derivative. An orally administered vitamin D derivative is selected from: dihydrotachysterol (1 mg daily), 1,25-dihydroxycholecalciferol (1 mcg daily), 25-hydroxycholecalciferol (0.1 mg daily), ergocholecalciferol (1.25 mg daily), and cholecalciferol (1 mg daily). Synthetic vitamin D derivatives are administered topically and is selected from the group consisting of calcipotriene and calcitriol (both at a concentration of 0.005% in an ointment or lotion or shampoo). These pharmacological compositions may be used to treat acute or chronic disease or may be used prophylactically to prevent the onset of the disease.

Example 13: Use of Pioglitazone as Synergistic (Adjunctive) Therapy in the Treatment of Multiple Sclerosis (MS) - A Clinical Trial

Current therapies for MS consist of three interferonβ preparations (Betaseron, Avonex, and Rebif), Copolymer 1 (Copaxone), and Novantrone. According to this invention, a PPARα and/or PPARγ agonist may be used adjunctively in combination therapy with any of the existing (approved) therapies (identified above) for treating MS. For the purpose of an example, rosiglitazone, pioglitazone, KRP 297, MCC 555 or JTT-501 for the stated adjunctive use at doses outlined above. The patient presents acutely with the neurological manifestations of multiple sclerosis, and the diagnosis is confirmed by clinical laboratory and pathological diagnostic tests. The patient is evaluated by a neurologist experienced in the clinical and laboratory manifestations of multiple sclerosis lesions. The patient is a male weighing 70 kilograms or a female weighing 50 kilograms and being treated with an interferonβ preparation, Copaxone, or Novantrone. An adjunctive PPARα agonist, a PPARγ agonist, or a PPARα and PPARγ co-agonist (co-activator), is selected from one of the following group of compounds:

1) PPARγ/PPARα ligand:

KRP 297: 7.5 mg to 50 mg QD or as split dose BID. Preferred dose 20 to 40 mg QD.

Bezafibrate: 25 mg to 200 mg QD or as split dose BID. Preferred dose 50 to 100 mg QD

MCC 555: 5 mg to 30 mg QD or as split dose BID. Preferred dose 5 mg BID.

JTT-501: 5 mg to 30 mg QD or as split dose BID. Preferred dose 5 mg BID.

Adjunctive therapy is initiated at one-half the preferred dose as indicated above. The

dose is doubled within 6 to 8 wks of initiation of said adjunctive therapy. The patient is monitored for improvement based on laboratory and clinical findings. The regime is continued as medically indicated to one of skill in the art of treating MS.

All publications, patents and patent publications mentioned in this specification are herein incorporated by reference into the specification in their entirety for all purposes. Although the invention has been described with reference to preferred embodiments and examples thereof, the scope of the present invention is not limited only to those described embodiments. As will be apparent to persons skilled in the art, modifications and adaptations to the above-described invention can be made without departing from the spirit and scope of the invention, which is defined and circumscribed by the appended claims.

The foregoing is offered primarily for purposes of illustration. It will be readily apparent to those of ordinary skill in the art that the operating conditions, materials, procedural steps and other parameters of the invention described herein may be further modified or substituted in various ways without departing from the spirit and scope of the invention. For example, the invention has been described with human patients as the usual recipient, but veterinary use is also contemplated. Thus, the preceding description of the invention should not be viewed as limiting but as merely exemplary.

Tables I through X in the attached appendix, which are incorporated in whole by this reference, provide further examples of diseases or disorders treatable with methods described in this invention.

WHAT IS CLAIMED IS:

1. A method for treating an inflammatory state, condition, and disease of mammalian tissues, organs or cells, in a host suffering therefrom, said method comprising administering to a mammal in need thereof a therapeutically effective amount of an insulin-sensitizing agent that inhibits cellular production of the pro-inflammatory cytokine, interleukin-2, or pharmaceutically acceptable salts, solvates, tautomers or stereoisomers thereof, formulated with a pharmaceutically acceptable carrier or excipient.

2. A method of claim 1 wherein said insulin-sensitizing agent is a PPARγ ligand, and is selected from the group consisting of: 1) a PPARγ agonist, 2) a PPARγ agonist that also activates PPARα (PPARγ/PPARα dual agonist), 3) a PPARγ agonist that also activates PPARδ (PPARγ/PPARδ dual agonist), 4) a PPARγ agonist that also activates both PPARα and PPARδ (PPAR "pan-agonist"), 5) a PPARγ partial agonist, 6) a PPARγ/RXR agonist.

3. A method of claim 1 wherein the inflammatory disease is selected from the group consisting of a dermatological disease, a neurodegenerative disease, a cardiovascular disease, a pulmonary disease, an ophthalmic disease.

4. A method of claim 2 wherein the inflammatory disease is selected from the group consisting of a dermatological disease, a neurodegenerative disease, a cardiovascular disease, a pulmonary disease, an ophthalmic disease.

5. A method of claim 2 wherein the insulin-sensitizing PPARγ agonist agent is a thiazolidinedione or derivative thereof, selected from the group consisting of rosiglitazone, rosiglitazone maleate, pioglitazone, troglitazone, ciglitazone, englitazone, darglitazone, NC-2100, KRP 297, MCC-555, JTT-501(and its metabolite, JTT-601), BM13.1258, BM15.2054.

6. A method of claim 5 wherein the thiazolidinedione is a 5-aryl-2,4-thiazolidinedione derivative.

7. A method of claim 1 wherein the insulin-sensitizing agent is an oxazolidinedione derivative or an isoxazolinedione derivative.

8. A method of claim 1 wherein the insulin-sensitizing agent is an alpha-methoxy-beta-phenylpropanoic acid derivative.

9. A method of claim 1 wherein the insulin-sensitizing agent is a N-(2-Benzoylphenyl)-

L-tyrosine derivative.

10. A method of claim 1 wherein the insulin-sensitizing agent is phenylacetic acid derivative.

11. A method of claim 1 wherein the insulin-sensitizing agent is an indole-based PPARγ agonist.

12. A method of claim 1 wherein the insulin-sensitizing agent is a cinnamic acid derivative or a dihydrocinnamic acid derivative.

13. A method of claim 1 wherein the insulin-sensitizing agent is a tricyclic-[alpha]-alkoxyphenyl-propionic acid derivative.

14. A method of claim 1 wherein said PPARγ partial agonist inhibits adipocyte differentiation and adipogenesis, an example being GW0072.

15. A method of claim 1 wherein the insulin-sensitizing agent is a RXR/PPARgamma agonist.

16. A method of claim 14 wherein the RXR/PPARgamma agonist is selected from the group consisting of LG100268, LGD100324, LG100754.

17. A method of claim 2 wherein the insulin-sensitizing agent is GW7845,GW1929, GW0207, GW0072, SB 213068, GW2433, GI262570 (also known as GW262570), GW409544, GW958, GW2433, GW 9578, GW2433, SB 213068, L-764406, L-165041, L-796449, DRF-4158, FK-614, DRF-2725 (NNC 61-0029), (-)DRF-2725,Arg (NNC 61-0029-arginine), NNC 61-4424, AZ 242 ((S)-2-Ethoxy-3-[4-(2-{4-methanesulfonyloxyphenyl}ethoxy)phenyl]propanoic acid).

18. A method of claim 2 wherein the EC50 for human PPARγ activation is within 3 orders of magnitude of the EC50 for human PPARα activation.

19. A method of claim 2 wherein the EC50 for human PPARγ activation is within 3 orders of magnitude of the EC50 for human PPARδ activation.

20. A method of claim 3 wherein the neurodegenerative disease is selected from the group consisting of Alzheimer's disease, Parkinson's disease, multiple sclerosis, stroke, and traumatic brain and spinal cord injury.

21. A method of claim 3 wherein the dermatological disease is selected from the group consisting of psoriasis, atopic dermatitis, allergic dermatitis, contact dermatitis, seborrheic dermatitis, solar dermatitis, acne rosacea, acne vulgaris, hidradenitis,

ichthiosis, warts, verrucae, eczema, sunburn, seborrheic keratosis, senile keratosis, photo-induced keratosis, skin aging, thinning skin, wrinkle formation, photo-induced skin aging, keloids androgenic alopecia, female hirsutism, with proviso that the PPARγ ligand is not a thiazolidinedione.

22. A method of claim 3 wherein the cardiovascular disease is selected from the group consisting of atherosclerosis, congestive heart failure, cardiomyopathy, hypertension, vascular stenosis, vascular restenosis.

23. A method of claim 3 wherein the pulmonary disease is selected from the group consisting of asthma, chronic obstructive pulmonary disease, reactive airway disease, pulmonary fibrosis.

24. A method of claim 3 wherein the ophthalmic disease is selected from the group consisting of corneal abrasion or ulceration, dry eye syndrome, pterygium/pinguecula, scleritis/episcleritis, glaucoma, uveitis, uveoretinitis, retinitis, retinitis pigmentosa, viral retinitis, chorioretinitis, choroiditis, vitreitis, diabetic retinopathy, hypertensive retinopathy, age-related-macular degeneration, white dot syndromes, optic neuritis, ischemic retinopathy, glaucomatous retinopathy, retinovascular retinopathies, choroidal retinopathy, age-related-macular degeneration, and neovascularization of the choroid, retina, subretina and iris.

25. A method of claim 3 wherein the insulin-sensitizing agent is rosiglitazone or rosiglitazone maleate, administered at a total daily dose of 8 mg, with the proviso that the inflammatory disease is not a dermatologic, neurodegenerative or cardiovascular disease.

26. A method of claim 3 wherein the insulin-sensitizing agent is pioglitazone administered at a total daily dose of 45 mg, with the proviso that the inflammatory disease is not a dermatologic, neurodegenerative or cardiovascular disease.

27. A method of claim 3 wherein the insulin-sensitizing agent is KRP 297 administered at a total daily dose of 1 to 50 mg, with the proviso that the inflammatory disease is not a dermatologic or cardiovascular disease.

28. A method of claim 3 wherein the insulin-sensitizing agent is MCC 555 administered at a total daily dose of 1 to 50 mg, with the proviso that the inflammatory disease is not a dermatologic or cardiovascular disease.

29. A method of claim 3 wherein the insulin-sensitizing agent is JTT-501 or its malonic

amide JTT-601, administered at a total daily dose of 1 to 50 mg.

30. A method of claim 4 wherein the insulin-sensitizing agent is AZ 242 administered at a total daily dose of 1 to 50 mg.

31. A method of claim 4 wherein the neurodegenerative disease is selected from the group consisting of Alzheimer's disease, Parkinson's disease, multiple sclerosis, stroke, and traumatic brain and spinal cord injury.

32. A method of claim 4 wherein the dermatological disease is selected from the group consisting of psoriasis, atopic dermatitis, allergic dermatitis, contact dermatitis, seborrheic dermatitis, solar dermatitis, acne rosacea, acne vulgaris, hidradenitis, ichthiosis, warts, verrucae, eczema, sunburn, seborrheic keratosis, senile keratosis, photo-induced keratosis, skin aging, thinning skin, wrinkle formation, photo-induced skin aging, keloids androgenic alopecia, female hirsutism, with proviso that the PPARγ ligand is not a thiazolidinedione.

33. A method of claim 4 wherein the cardiovascular disease is selected from the group consisting of atherosclerosis, congestive heart failure, cardiomyopathy, hypertension, vascular stenosis, vascular restenosis.

34. A method of claim 4 wherein the pulmonary disease is selected from the group consisting of asthma, chronic obstructive pulmonary disease, reactive airway disease, pulmonary fibrosis.

35. A method of claim 4 wherein the ophthalmic disease is selected from the group consisting of corneal abrasion or ulceration, dry eye syndrome, pterygium/pinguecula, scleritis/episcleritis, glaucoma, uveitis, uveoretinitis, retinitis, retinitis pigmentosa, viral retinitis, chorioretinitis, choroiditis, vitreitis, diabetic retinopathy, hypertensive retinopathy, age-related-macular degeneration, white dot syndromes, optic neuritis, ischemic retinopathy, glaucomatous retinopathy, retinovascular retinopathies, choroidal retinopathy, age-related-macular degeneration, and neovascularization of the choroid, retina, subretina and iris.

36. A method of claim 4 wherein the insulin-sensitizing agent is rosiglitazone or rosiglitazone maleate, administered at a total daily dose of 8 mg, with the proviso that the inflammatory disease is not a dermatologic, neurodegenerative or cardiovascular disease.

37. A method of claim 4 wherein the insulin-sensitizing agent is pioglitazone

administered at a total daily dose of 45 mg, with the proviso that the inflammatory disease is not a dermatologic, neurodegenerative or cardiovascular disease.

38. A method of claim 4 wherein the insulin-sensitizing agent is KRP 297 administered at a total daily dose of 1 to 50 mg, with the proviso that the inflammatory disease is not a dermatologic or cardiovascular disease.

39. A method of claim 4 wherein the insulin-sensitizing agent is MCC 555 administered at a total daily dose of 1 to 50 mg, with the proviso that the inflammatory disease is not a dermatologic or cardiovascular disease.

40. A method of claim 4 wherein the insulin-sensitizing agent is JTT-501 or its malonic amide JTT-601, administered at a total daily dose of 1 to 50 mg.

41. A method of claim 4 wherein the insulin-sensitizing agent is AZ 242 administered at a total daily dose of 1 to 50 mg.

INTERNATIONAL SEARCH REPORT

Inter ial application No.
PCT/US01/25668

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 31/19, 31/42, 31/44, 31/47, 31/50, 31/195, 31/425, 31/495

US CL :514/249, 312, 369, 352, 354, 374, 375, 470, 562, 569, 570

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/249, 312, 369, 352, 354, 374, 375, 470, 562, 569, 570

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

REGISTRY, HCAPLUS, USPATFULL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 5,981,586 A (PERSHADSINGH) 09 Nov 1999, abstract, claims.	1-6, 15, 18-19, 21, 32 --- 7-14, 16-17, 20, 22-31, 33-41
X --- Y	US 6,034,110 A (NAGPAL et al) 07 March 2000, abstract, claims.	1-6, 15, 18-19, 22, 33 --- 7-14, 16-17, 20-21, 23-32, 33-41

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)		
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search 20 DECEMBER 2001	Date of mailing of the international search report 25 JAN 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer REBECCA COOK Telephone No. (703) 308-1235

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